Abnormalities in hylid frogs: a case study of schizodactyly in the striped snouted treefrog, Scinax squalirostris (Lutz, 1925) (Amphibia: Anura: Hylidae)

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Abstract: Abnormalities in amphibians have been reported around the world in a broad number of taxa (e.g., Mahapatra et al., 2001; Piha et al., 2006; Dias and Carvalho-e-Silva, 2012; Wagner et al., 2015). The terminology used to describe the diversity of morphological abnormalities was defined by Henle et al. (2017) and followed by us in the present work. Many factors influence the development of morphological abnormalities in amphibians, such as genetic predisposition (Droin and Fischberg, 1980; Correia et al., 2018), heavy metal concentrations (Zocche et al., 2013; Huang et al., 2014), hyperregeneration following trauma (Van Valen, 1973; Thomson et al., 2014), radiation exposure (Blaustein et al., 1997), diseases and parasites (Sessions and Ruth, 1990; Sessions et al., 1999; Kiesecker, 2002), and habitat pollution by agricultural pesticides (Ouellet et al., 1997; Sparling et al., 2015; Koleska and Jablonski, 2016), with the last factor has being identified as the major cause for such malformations (Lunde and Johnson, 2012). Synergistic interactions of some of these factors may also influence malformations (Kiesecker, 2002; Johnson et al., 2006). Rates of abnormalities are considered natural when present in less than 5% of the population (Stocum, 2000).

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Abnormalities in amphibians have been reported around the world in a broad number of taxa (e.g., Mahapatra et al., 2001; Piha et al., 2006; Dias and Carvalho-e-Silva, 2012; Wagner et al., 2015). The terminology used to describe the diversity of morphological abnormalities was defined by Henle et al. (2017) and followed by us in the present work.

Many factors influence the development of morphological abnormalities in amphibians, such as genetic predisposition (Droin and Fischberg, 1980; Correia et al., 2018), heavy metal concentrations (Zocche et al., 2013; Huang et al., 2014), hyperregeneration following trauma (Van Valen, 1973; Thomson et al., 2014), radiation exposure (Blaustein et al., 1997), diseases and parasites (Sessions and Ruth, 1990; Sessions et al., 1999; Kiesecker, 2002), and habitat pollution by agricultural pesticides (Ouellet et al., 1997; Sparling et al., 2015; Koleska and Jablonski, 2016), with the last factor has being identified as the major cause for such malformations (Lunde and Johnson, 2012). Synergistic interactions of some of these factors may also influence malformations (Kiesecker, 2002; Johnson et al., 2006). Rates of abnormalities are considered natural when present in less than 5% of the population (Stocum, 2000).

Abnormalities in anurans of the family Hylidae have been reported for both larval (e.g., Sayim and Kaya, 2006; Silva and Toledo, 2010; Peltzer et al., 2013; Natale et al., 2018) and adult individuals (e.g., Lannoo, 2008) and include several types of morphological alterations. Besides abnormal colour patterns (e.g., Henle et al., 2017; Sousa and Costa-Campos, 2017), there are records of sacrum asymmetric in *Boana geographicus* (Peloso, 2016); anophthalmy in *Dendropsophus luddeckei*, *Boana faber* (both eyes), *Boana fasciatus* and *Osteocephalus leprieurii* (Ramalho et al., 2017; Brassaloti and Bertoluci, 2018; Suárez, 2018); gonadal malformations in *Scinax fuscovarius* (Goldberg, 2015); brachydactyly, syndactyly, and ectromely in *Isthmohyla rivularis* (Hedrick and Cossel Jr, 2014); polymely in *Hyliola regilla* and *Acris crepitans* (Reynolds and Stevens, 1984; Sessions and Ruth, 1990; Gray, 2000; Johnson et al., 2003; McCallum and Trauth, 2003); ectrodactyly in *Pseudacris streckeri* (Gridi-Papp and Gridi-Papp, 2005); brachydactyly (Ectrodactyly) in *Corythomantis greening* (Silva-Soares and Mônico, 2017); and several hind leg malformations (mostly amely) in North American Hylids suchlike *Acris crepitans*, *A. gryllus*, *Dryophytes arenicolor*, *D. chrysoscelis*, *D. cinereus*, *D. femoralis*, *D. squirellus*, *D. versicolor*, *D. wrightorum*, *Osteopilus septentrionalis*, *Pseudacris cadaverina*, *P. crucifer*, *P. feriarum*, *P. maculata*, *P. ocularis*, *P. ornata*, *P. regilla* and *P. triseriata* (Lannoo, 2008). Despite the vast knowledge regarding different types of anomalies in Hylidae, we failed to find any record of schizodactyly (following Henle et al. (2017): "especial case of polydactyly in which the proximal parts of the duplicated digit are fused") in this family. We reviewed all cases of polydactyly with special care to avoid misconceptions due to the usage of different terminologies, but we found either different types of anomalies or inconclusive information. Therefore, to our knowledge, this is the first formal report of schizodactyly in the Hylidae family.

During a nocturnal survey in Botucatu Municipality in the rural area of São Paulo state, southeastern Brazil, on 22 March 2015, we collected an adult male specimen of *Scinax squalirostris* presenting an extra phalanx on left toe IV (Fig. 2a). The frog was found vocalizing on top of a grass leaf of a highly environmentally

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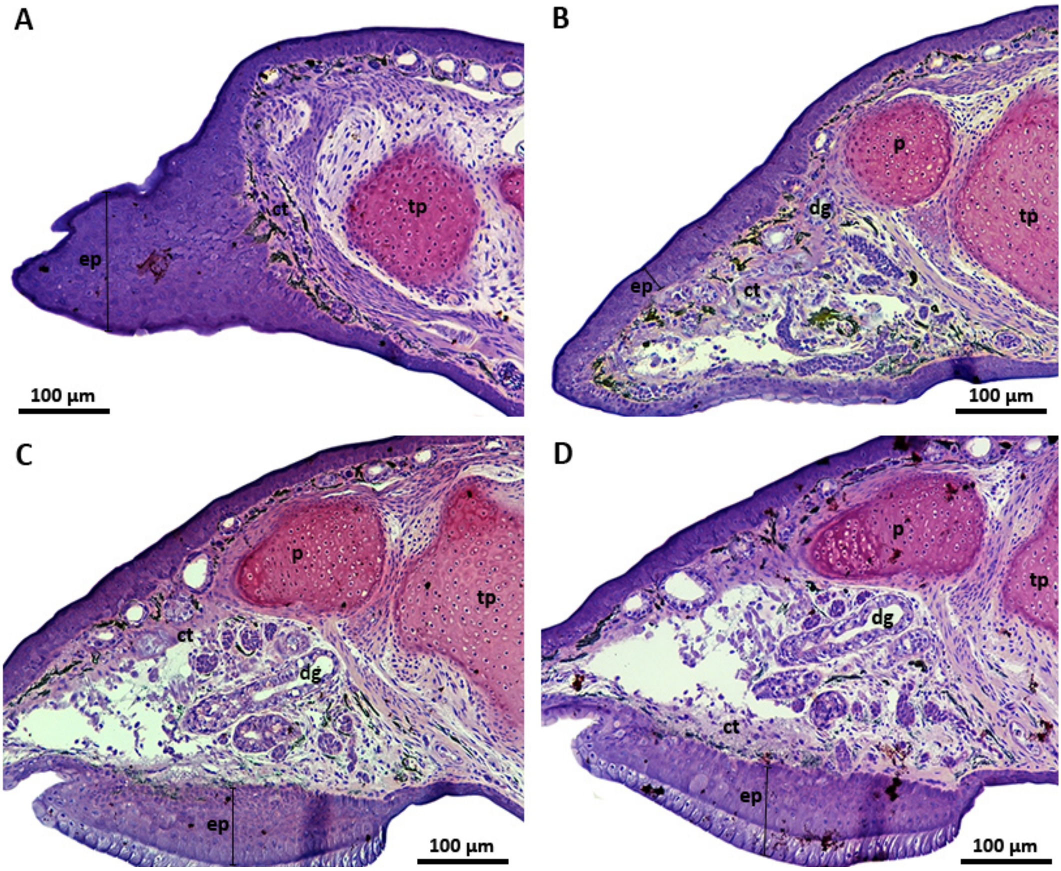

Figure 1. Light micrographs of the extra finger stained with toluidine blue/basic fuchsin. (A) Notice the lateral expansion between the toe's IV medial and proximal phalanx. (B, C, D) Notice the phalanx of the extra finger closer to the dorsum of the hind limb and a thick layer of connective tissue with forming glands below the bone structure as sections progress. ep., epidermis; ct., connective tissue; dg., developing glands; tp., toe's phalanx; ep., extra finger's phalanx.

impacted swamp area close to the city (22°56'20.4"S, 48°27'36.8"W). The anomaly did not seem to impair the individual's climbing abilities and was only noticed during specimen fixation. The individual was deposited at the Museum of Zoology of the University of São Paulo (MZUSP158735).

We assessed the bony structure as well as the soft tissue arrangement through the usage of Micro-computed tomography (micro-CT) and histological techniques. Scanning was performed in 360º with at least 1061 images per scan, with x-ray operating at 43 kV and exposure time of 750 ms (Fig. 2b), using a SKYSCAN 1176 in viva microtomograph. We then carefully dissected the abnormal toe from the rest of the foot and dehydrated the sample in an ascending series of ethanol following the procedures for histological examination. The sample was resin-embedded, sectioned transversely at 4.5 µm and mounted onto microscope slides. Sections were stained with toluidine blue/basic fuchsine for general histology (Junqueira, 1995).

The histology of toe IV was analysed alongside the histology of the extra phalanx and observations showed that both structures are similar. Between the medial and proximal phalanx of toe IV we observed a lateral expansion of connective tissue and epidermis forming the extra phalanx (Fig. 1a). As sections progress, a new phalanx can be seen in this projection, composing its bone structure (Fig. 1b-d). We observed a thin layer of connective tissue between the phalanx of the toe IV and the phalanx of the extra finger (Fig. 1b-d). When compared to the position of the toe's IV phalanx, the extra finger is closer to the dorsum of the hind limb and

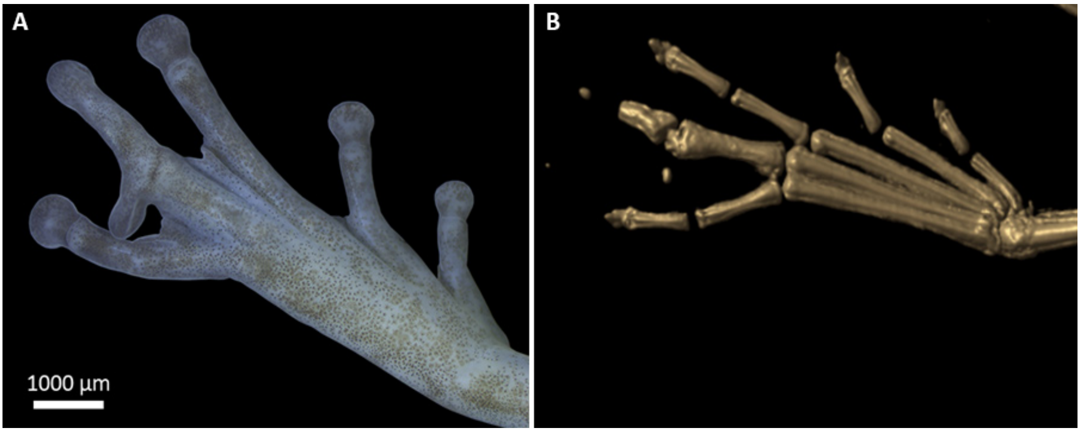

Figure 2. Dorsal view of schizodactyly on the toe IV of the left hind limb in *Scinax squalirostris*. (A) External morphology showing all toes, including the extra finger described in this study. (B) CT scan showing the bone morphology of all toes and including a bony structure of the extra finger.

a thicker layer of connective tissue with developing glands and epidermis is visible below the extra phalanx (Fig. 1b-d). Epidermis is thin, with a keratinized outer layer and gland ducts opening to the exterior as generally seen in anuran's epidermis (Brunetti et al., 2012).

We were not able to infer the cause of the abnormality. Although *Scinax squalirostris* is known to be more sensitive to chemicals, such as chromium, than some other anuran species (Natale et al., 2000), no other malformed frogs were seen at this locality. We thereby suggest that this case of schizodactyly probably resulted of natural causes. Genetic mutations associated with limb and digit development, such as BmP, FgF Hedgehog, and Hox, have been reported to induce this kind of malformation (Droin and Fischberg, 1980; Correia et al., 2018). However, further investigation is required regarding the underlying processes driving this specific malformation.

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